

Allosteric effects in haemoglobin

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absorption spectrum in the visible region or in the near ultraviolet region (Soret region) is recorded (Fig. 4.1). A small volume of air is injected into the tonometer, the liquid and gas phases are equilibrated at a constant temperature, and another spectrum is recorded. This procedure is repeated until the haemoglobin is fully oxygenated. Y is calculated from absorbance changes at any wavelength as follows,

$$Y = \frac{A - A_{\text{deoxy}}}{A_{\text{oxy}} - A_{\text{deoxy}}} \quad (4.1)$$

Here, A_{oxy} and A_{deoxy} are the absorbance of the fully oxygenated sample and the fully deoxygenated sample, respectively, and A is the absorbance at a given pO_2 . This method is based on an assumption that the degree of change in absorbance of a haemoglobin solution at any fixed wavelength is proportional to Y , i.e. the ratio of oxygenated haems to total haems in the solution. Although this assumption has been accepted empirically, and there is no doubt about its validity for practical use, its strict validity is subject to question for certain reasons (refer to § 4.5.1). Dual-wavelength spectrophotometry is used for turbid samples such as whole blood or red cell suspensions.

Fig. 4.1 Light absorption spectra of oxyhaemoglobin (—), deoxyhaemoglobin (---), and aquomethaemoglobin (···) in the visible and near ultraviolet regions. E_{mM} is millimolar extinction coefficient in 0.1 M potassium phosphate buffer (pH 7.4).

